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(54) Regulated genes by stimulation of chondrocytes with 1L-1beta

(57) The present invention refers to the novel use of osteopontin, calnexin and TSG-6 gene product in the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1 β and their use in the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues.

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Description

The present invention refers to the novel use of osteopontin and calnexin in the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1 β and their use in the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues.

Among the diverse biological effect of interleukin-1 (IL-1), are its actions on the metabolism of many connective tissue cell types including articular chondrocytes. IL-1 inhibits proteoglycan (PG) synthesis by chondrocytes and stimulates production of prostaglandin E₂ and metallo-proteinases capable of degrading matrix macromolecules. From experimental results, and from findings of IL-1, PG fragments and proteolytic enzymes in inflamed joints, it was deduced that IL-1 plays a role in cartilage degradation in osteoarthritis and rheumatoid arthritis (Benton HP & Tyler JA. 1988, Biochem, Biophys. Res. Comm. 154, 421-428; Aydelotte MB et al. Conn. Tiss. Res. 28, 143-159; Wood DD et al., Arthritis Rheum. 26, 975-983; Lohmander LS et al., Trans Orthop. Res. Soc. 17, 273). Matrix metalloproteinases are potential candidates for drug interaction at the enzyme level, but relevant molecular targets interfering with earlier processes leading to cartilage degradation are still lacking. Therefore, one objective of the present invention was to identify potential targets for drug modification of IL-1 β induced cartilage degradation on the RNA level of human articular chondrocytes from osteoarthritic cartilage.

As an initial attempt to investigate differentially expressed genes in diseased cartilage, total RNA from IL-1 β stimulated and unstimulated human chondrocytes was subjected to differential display of mRNA by reverse transcription and polymerase chain reaction (DDRT-PCR). This method can be used to identify and isolate those genes that are differentially expressed in two cell populations (Liang P & Pardee AB 1992, *Science* 257, 967-971; Liang P et al., AB 1993, *Nucl. Acids Res.* 21, 3269-3275; Bauer D et al. 1993, *Nucl. Acids Res.* 21, 4272-4280). The key element is to use a set of oligonucleotide primers, one hybridizing to the polyadenylated tail of mRNAs, the other being arbitrary decamers that anneal at different positions relative to the first primer. mRNA subpopulations defined by these primer pairs are amplified after reverse transcription and resolved on DNA sequencing gels. Band patterns are created, which are characteristic for each RNA population extracted from the cell population under study. For example, 100 different primer combinations should generate a total of approximately 10,000 PCR products for each population, which should represent about the half of all expressed cellular genes. A comparison of the band pattern obtained from two cell populations reveals differentially displayed bands which correspond to differentially expressed genes. Subsequently, differentially displayed bands can be extracted from the gel, reamplified, subcloned and sequenced.

30 Due to its extreme sensitivity, the appearance of artifactual bands is an inherent problem of the DDRT-PCR method used according to the present application. An additional problem is also the evaluation of complex gene expression patterns. Yet another problem of the present invention is that only minute amounts of RNA are available.

Therefore, it was particularly surprising that the DNA TAU1/1 with the sequences

35 TAU1/1(1)
ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC ATCCCCGTTT 60
CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG GAAGGACAGT TATGAAACGA 120
40 GTCAGCTGGA TGACCAAGAGT GCTGAAACCC ACAGCCACAA GCAGTCCAGA TTATATAAGC 180
CGAAA 185

45 and
TAU1/1(2)
CTAAATGCAA AGTGAGAAAT TGTATTTTT CTCCTTTAA TTGACCTCAG AAGATGCACT 60
50 ATCTAATTCA TGAGAAATAC GAAATTCAG GTGTTTATCT TCTTCCTTAC TTTTGGGG 118

and the DNA TTU2/2 with the sequence

5	AACCACTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT TGTTTCTTA	60
	TCAGTA ³ AAT AGGTCTTCAG ATCTGCATCT GCCCTCTTAG CATGTTTTC TTCATAGATA	120
	CCCGTTTUG GGTTTTGCCG TCGGAAGATG AATGGCATT ATAGTCCTCT CCACATTTAT	180
	CTG	183

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are 100 % identical to human osteopontin cDNA and 97.2 identical to human calnexin, respectively. This demonstrates that the experimental approach of the present invention worked efficiently, i.e. the use of 100 different primer combinations (25 oligodecamer primers, 4T₁₂MN-primers) generated a total of approximately 10.000 PCR products for each population which represent 53 % of all expressed cellular genes. 123 PCR bands out of 10.000 appeared as differentially expressed bands. 53 of the original 123 PCR bands were reproducibly displayed by comparing the PCR band patterns from two patients; of those 68 % arose from IL-1 β stimulated chondrocytes.

It was further found that osteopontin which is a secreted highly acidic phosphoprotein of 32 kd (Denhardt and Guo (1993) FASEB J. 7, 1475-1482) is surprisingly downregulated in IL-1 β stimulated human chondrocytes. This means that osteopontin is involved in IL-1 β related diseases of connective tissues, in particular osteoarthritis.

20 Osteoarthritis is characterized as a slowly progressing matrix degeneration with continuing degradation of collagens and proteoglycans and subsequent release of matrix fragments into the synovial fluid. Any disturbance of the normal chondrocyte matrix interactions, for example through a loss of osteopontin, could cause an altered signaling through the integrin alpha₁beta₁ and thus changed cellular responses leading to early steps of matrix degradation.

25 Therefore, one embodiment of the present invention is the use of osteopontin itself or parts thereof, antibodies against it or nucleic acids such as DNA or RNA or parts thereof coding for osteopontin or parts thereof in the diagnoses, prophylaxis or therapy of IL-1 β related diseases of connective tissues, in particular osteoarthritis. According to the present application the term "parts" means either at least 8, preferably 12, in particular 15 amino acids in case of proteins or 6-100, preferably 10-40, in particular 12-25 nucleic acids in case of DNA or RNA as hybridization probes. The methods of diagnosing such diseases will be described infra. In addition, quantification on the protein level is possible with osteopontin specific antibodies on Western blots, in immunochemistry, FACS analysis or ELISA based assay systems. The present invention refers also to a diagnosis aid or a pharmaceutical for such use. Osteopontin can be produced for example recombinantly through expression in procaryotes, in insect cells in mammalian cells or in mammalian cells using Vaccinia as detailed in Ausubel et al. 1994 [Current protocols in molecular biology, Chapter 16, John Wiley & Sons, Inc]. The cDNA of Osteopontin is e.g. disclosed in Young et al. (1990), Genomics 7, 491 - 502.

35 Antibodies against osteopontin can be generally produced for example by the method of Neil GA & Urnovitz HB (Trends in Biotechnology, 6, 209-213, 1988) or Köhler G & Milstein C (Nature, 256, 52-53, 1975).

Also calnexin which is an integral membrane protein of 88 kd (Bergeron et al. (1994) TIBS 19, 124-128) is surprisingly downregulated in IL-1 β stimulated human chondrocytes compared to unstimulated chondrocytes. This means also that calnexin is involved in IL-1 β related diseases of connective tissues, in particular osteoarthritis. In addition, a downregulation of the calnexin synthesis would cause a reduced amount of correctly and completely folded proteoglycans because calnexin is a new type of molecular chaperone that associates with incompletely folded proteins such as proteoglycans. Proteoglycans are highly glycosylated glycoproteins which are of central importance for the maintenance of the cartilage tissue integrity.

40 Hence, an additional embodiment of the present invention is the use of calnexin itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 β related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

45 Calnexin can be produced for example recombinantly as described above for osteopontin. The cDNA of Calnexin is e.g. disclosed in Galvin et al. (1992), Proc. Natl. Acad. Sci. USA 89, 8452 - 8456. The production of said antibodies are also generally described above.

Potential role of identified cDNA fragments in IL-1 mediated cellular processes TSG-6

50 55 A homology search in the GenBank and EMBL databases revealed a 99.5 % sequence identity of fragment TAU7/2(c) with the gene coding for human TSG-6. TSG-6 (TNF stimulated gene 6) was originally isolated by differential cDNA library screening as a TNF induced gene sequence from human fibroblasts (Lee et al., 1990). It was further characterized by Lee et al (1992) as a TNF and IL-1 inducible, secretory, 39 kDa glycoprotein with extensive sequence homology with a region implicated in hyaluronate binding, present in cartilage link protein, proteoglycan core proteins,

and the adhesion receptor CD44. With the ability to bind HA and with the most extensive sequence homology to CD44, TSG-6 belongs to the hyaladherin family. Wisniewski et al. (1993) detected high levels of TSG-6 protein in synovial fluids of patients with various forms of arthritis. Six normal control patients did not contain detectable TSG-6 protein in their joint fluid, whereas joint fluids from nine rheumatoid arthritis patients contained high, moderate or low levels of TSG-6.

5 Two patients with osteoarthritis had high levels of TSG-6 in their joint fluids. The apparent local source of TSG-6 in the joints are synoviocytes and chondrocytes (Wisniewski et al., 1993). Lee et al. (1992) speculated that TSG-6 could act as a competitive inhibitor of the interaction between CD44 and its ligand(s) and thus might influence the structural organization of the extracellular matrix of connective tissue, resulting in a destabilization of the proteoglycan aggregates.

Hence, an additional embodiment of the present invention is the use of TSG-6 gene product itself, or parts thereof
10 antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 β related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

15 Fibronectin

A homology search in the GenBank and EMBL databases revealed a 100 % sequence identity of fragment TTO20/1(c) with the gene coding for human fibronectin.

Fibronectin is a 450 kd glycoprotein with various functions. It acts as an adhesive ligand, as growth or differentiation factor and has chemotactic properties. It is found in the extracellular matrix of most types of cells (Hynes R 1993. Fibronectins, In: Guidebook to the extracellular matrix and adhesion proteins. Editors: Kreis T, Vale R. Oxford University Press. 56-58). An enhanced accumulation of fibronectin and fragments derived from it are found in the synovial fluid and on the inflamed synovial and pannus surfaces in the knee joint of patients with rheumatoid arthritis (Dutu A, Vlaicu-Rus V, Bolosiu HD, Parasca I, Cristea A. 1986. Fibronectin in plasma and synovial fluid of patients with rheumatic diseases. Med. Interne 24, 61-68). Patients with osteoarthritis, as well, have greatly increased levels of fibronectin in their synovial fluid and on cartilage surfaces (Xie D-L, Meyers R, Homandberg GA. 1992. Fibronectin fragments in osteoarthritic synovial fluid. J. Rheumatology 19, 1448-1452). The intraarticular injection of fibronectin fragments causes a severe depletion of cartilage proteoglycans in vivo (Homandberg GA, Meyers R, Williams JM. 1993. Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans in vivo. J. of Rheumatology 20, 1378-1382), which is explained by the induced release of several proteinases, including stromelysin (Xie D-L, Hui F, Meyers R, Homandberg GA. 1994. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: Stromelysin plays a major role in chondrolysis. Arch. Biochem. and Biophysics 311, 205-212). At high concentrations, fibronectin fragments enhance cartilage catabolism through release of cytokines, including IL-1 (Homandberg et al., personal communication).

35 In respect to these published data, the upregulation of fibronectin by IL-1 can be regarded as a positive feedback regulation, enhancing the self destructive potential of chondrocytes and synoviocytes. With this, fibronectin expression is a direct pharmacological target.

In addition, the sequencing of differentially displayed PCR products discovered also unknown DNA fragments which correspond to differentially expressed genes with or without stimulation of chondrocytes with IL-1 β .

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Therefore, another embodiment of the present invention is a DNA containing a DNA selected from the group consisting of

5 TA08/2 (2)

1	CCAAGTTTT	CCAGCAACCC	CAAGGGAAATA	CAGGGAGATC	AATGCACCA
51	AAATGGGAAA	AGAAAAAATAC	TTCGATGCAA	TGAAACAAAG	CCTTTTCCG
101	TTCAGTTCC	ATAATTCACT	GGTCAGTTT	AAGGCTGCCA	CTTGGG

10 TA016/1 (2)

1	GACACGAACA	CCACATATTT	TTATTGGAGG	CCCCATGGCT	CCTTGGAAAGC
51	CATTTGGAA	CCAAGGGGAC	CCACCTTTT		

15 TA016/2 (2)

1	CTAAATATAT	TCTCTAACAA	GTAAATCTCT	TTCAAATCTA	TAGATAAAAC
51	TAAAAGGATA	AGGAACCAAG	GTTTAACCGA	CCTAGCCAAT	TATGGCAATC
101	ATACTTGCTT	TTTAG			

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TA017(C)

5 1 CATGAAATAT TTCTTGAGGT AATAAGCTTT TACCAAGCTT ATATTTTTGG
 51 GCAATTCACT TACAATGAGA AAAAAACACA CCAAAAGACC AAAAATTTTA

 101 AAAACTCACT TTTCTTGCCT TCATAGACAT TTGCATTATT ATAGAACATT
 151 CAAACAAGTT AGGTGGATAA TTATTGTCTA TAGATAAATA CGATGCAATT
 201 TTTTTAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA
 301 AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTAAC
 351 AGATATGATG ACAGNAAAAA TTGATTGCAT ATCTTTTGC ACTAAAACCT
 401 TTATATTTAT TT

15 TA019(C)
 1 AGACCGAGGGG TATTCNCGG TTCATACCGC CATGGCTTAA GAAGCAAAAG
 51 TCATATACCT TAGTAGGGC AAAGATNGAG GAGATAAAA AGAGCCTACC
 20 101 CAAGCTGTTG TTGAAGAACCA GGTCTTAGAT AAAGAGGAAC CCTTCCAGAA
 151 GNACAGAGAC AGGCTAAGGG TGATGCTGAG GAAATGGCTC AGAAGAAACA
 201 AGAGATTAA

TAU 1/1(2)

25 1 CTAAATGCCTA AGTGAGAAAT TGTATTTTTT CTCCTTTAA TTGACCTCAG
 51 AAGATGCACT ATCTAATTCA TGAGAAATAC GAAATTCAG GTGTTATCT
 101 TCTTCCTTAC TTTTGGGG

30 TAU 1/1(1)
 1 ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC
 51 ATCCCCGTTT CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG
 101 GAAGGACAGT TATGAAACGA GTCAGCTGGA TGACCAGAGT GCTGAAACCC
 35 151 ACAGCCACAA GCAGTCCAGA TTATATAAGC GGAAA

TAU1/2(2)

40 1 CCGGAATGGG GAGCAAACCA TAAGAACCGG GACCAGTTTC CTCTCTTTGT
 51 GCCCTAGTTC CCCCTCCTTT GTATACACCC TCCATCCTGA ATAGACTCTG
 101 GTTCTCAGCG TAACACCGAC AACATTCAAT CCTGTAGAGA AACAAATGTT
 151 AGCTCAGAAG GACACAGCCT TTGAATCATC AGAGAGTT

TAU 7/1(2)

45 1 GTAAAGAATA ACTAAATAAA AGTTTTAATT AATTTAGGAA TATAAAAAAC
 51 TATTAACATT TAATTTTATA ACTGTATCTG CCAAGCAACT TTAAATATAA
 101 TTTATTTACC

50 TAU 7/1(1)
 1 CACGCAATGT GAAATAGGCA CATAGGAAGA ATGGGGAAAC CATCCCCCTCA
 51 AGCATTATC CTTTGAGTTA CAAGCAATCC AATTACACTC TTTTAGTTAT
 101 TTTAAATGT ACAGTTAGGT TATTA

TAU 7/2 (C)

5	1	CCTTGAAGAT	GACCCAGGTT	NCTTGGCTGA	TTATGTTGAA	ATATAGACA
	51	GTTACGATGA	TGTCATGGC	TTTGTGGAA	GATACTGTGG	AGATGAGCTT
	101	CCAGATGACA	TCATCAGTAC	AGGAAATGTC	ATGACCTTGA	AGTTTCTAAG
	151	TGATGCTTCA	GTGACAGCTG	GAGGTTCCA	AATCAAATAT	GTTGCAATGG
10	201	AT				

TAU10(1)

15	1	GGAGATGACA	TTTGCTTTGG	GCAGAGGCAG	CTAGCCAGGA	CACATTTCCA
	51	CTATAATTTC	ACAAAGTTAA	ATTTATAAGC	TAGCATTAAAG	TAAAGTGAAG
	101	TTCCAGCTCC	CTTGCTAAAA	ATAACTAGAG	GTAATAATTG	GTATTCAAGT
	151	AACTCATTAA	CATCATAATG	TGTTGTGAAA	A	

TAU12/1(2)

20	1	TATAAAATAT	AAATTATATT	ATAAATCATG	TATTATTTAT	AAAATTATAT
	51	TATAAAATTAA	AAAAAATATA	AATTATATT	TAGGCTTAAT	GTATAAGGAA
	101	TATAAAATTAT	TAATAAGCAT	ATGA		

TAU 12/1(1)

25	1	TGTAATTAAC	TGTCCTTGTA	GGTCTGTCTT	TTATACATGT	GTGAGTTTT
	51	CTTTACATAA	GATTCCTAGC	ATTGGGATTG	CTAGGTCAAGA	TGGTATGCAC
	101	ATTTGACATT	TTGATTGATA	GCACCAAGATT	GCTTGTAA	AAAATTTNN
	151	TTTATAGTTT	ACATTATCTT	TGTACAATAG	ATGTTCTCTT	TCGAC

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TAU 12/2(1)

35	1	GGGAAGTGA	TTGAAAATAC	TTCTTTNTCA	ACATAATTTT	NGGGTTTGA
	51	AATTGTGTTT	GGGTTTCAG	GAAATTGGTG	GTAATCTTGT	ATTAGACTGAA
	101	AAAAAGTGA	TTTAAAATT	CTCAGTGAAG	AAGCAAATGA	TTTATTTTC
	151	ATAGA				

TAU12/3(2)

40	1	TGTTCTGGTA	ACTGTTCTAA	TTGTGTCTTT	GTACTTCCA	GTGCAACCCCT
	51	TTCAGGTAAG				

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TAU12/3(1)

	1	CTAAAGAACT	TGGTATCTCT	ATTAAGCAC	ACGAACCTCC	AAGGAAAATA
	51	GAGCGATTAA	CTCTTCTCAT	ATCAGTGCAT	ATTTATAAGA	AGCACGGAGT
	101	CA				

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TAU13/1(1)

	1	AGTCATCAAT	TCCTTTTAT	CTGTAATTAC	ACATTTGTTT	TTATTCAAA
	51	GTAATTATAA	GGTGTATAT	TGCATATAAT	CAGAAAACTA	AATGAAATA
	101	AAATTTAGT	AAGCCCGGCC	CCTTGACCG	ATACAGAAAA	CTTGA

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TAU 13/3(2)

5	1	TATATGGCAG	TCTAAACCAT	CAAAGATTTG	CATCAACATC	TTTCATTTA
	51	GACATCTCCT	TGCAATGTAA	AATATCATGT	ATCAACAACA	TCTGGTGCAA
	101	ATCCATGAGT	CTAACTCGAC	ATTCATCTTA	GCTCGATTAT	TATTCCCTCG
	151	TACAGTCGAT	GTAAACAATA	CAGAAAGAGG	ATTATTAAGA	ACAGTTT

TAU 13/3(1)

10	1	ATTCATGAAA	TGGTCTATAT	GCATGATATT	GTAAATTCGG	ACTCGAAACC
	51	GAAACCAAGG	ATTCCGTTAC	AAAAATTCTC	TAATGCTGAC	AATGTTCTCA
	101	CGCAAACAAC	ATCATGGACA	TTAAATTCAA	GATATGTGAA	TGTTAATTCT
	151	GTCAATAAAG	TCAACGTAAA	GAGTAAAGTT	AAAAACAGTT	ATATCTNNNC
15	201	TGTCAATGAT	GAGTTAGTT	TAACAGATGA	TGAATCAATT	CT

TCO 16/1(C)

20	1	CAAAGTGT	TTGGTTTGA	GAGAGAGAGA	GATTGAGAGA	CAGAGAGAGA
	51	GAGAGAAACC	AAGGGATCAT	GATAGTTATA	GTCAAATACG	AGGTTGGATT
	101	ATCTTTGAA	AATGTGTTGG	TTCTGTGATA	CAAGAGGAAG	CTAAGACATA
	151	TCGTGGAAAC	ATCTCCCCC	TCCACCTTAA	TATCAAGAAC	AAATTGTGGA
	201	ATCTAATGTT	AATGAGAAGT	AGTTCCCCAC	TGTGTCAGAT	G

TCO16/2(C)

25	1	NCATCTGACA	CAGTGGGAA	CTACTTCTCA	TTAACATTAG	ATTCCACAAT
	51	TTNNNCTTGA	TATTAAGGNN	NNNNNGGAG	ATCGTTTCAC	GATATCGTCT
	101	TAGCTTCCTC	TTGTATCACA	GAACCAACAC	ATTTCAAAAG	ATAATCCTTC
	151	CTCNNTTGA	CTATAACTAT	CATGATCCCT	TGGTTCTCTC	TCTCTCTCTG
30	201	CTCTCTCATC	TCTCTCTCTC	TNAAAACNAA		

TCO17(C)

35	1	ACAGTAGTTA	GGAGTTCTT	TACTTACAAA	ATCACTGGAA	ATGATTAAT
	51	TGCTTTCCC	CCTCCCCAGA	GGTGCATTTT	TCTTATTTC	ATATAGTAAA
	101	GTTGAGCTTT	TACAGTCAT	AATGTGACAT	TTGGAATGCT	TATCAACTGC
	151	ATGTAAACAT	TAATAACCT			

TCO18(C)

40	1	GTAAATGGTA	TTANNNGCTG	AAGAAAAAAA	ATTTTCAAG	ACCTCTGTT
	51	TTTAACGTAA	CTTTATCATT	GGCATTGTGG	GCTTTGAAGT	TGCTGGGATA
	101	AATTAATATA	ATTAATAAAA	AGACTGAATT	TAATTGCAAA	AAAAAAAAAA
45	151	AACAAATAAGT	GTGGTGAT			

TCU2/1(1)

50	1	AAGAAATTAT	CCAGTTATT	ACAAGGCCAC	TGATATTTA	AACGTCCAAA
	51	AGTTGTTTA	AATGGGCTGT	TACCGCTGAG	AATGATGAGG	ATGAGAATGA
	101	TGGTTGAAGG	TTACATTAA	GGAAATGAAG	AAACTTAGAA	AATTAATATA
	151	AAGACAGTG	TAAATACAAA	GAAGATTT		

TCU2/2(1)

5	1	CGGGTTAATA	TTATCCTCTA	GTATAAGTGA	ATTACTAGTT	TCTCTTATT
	51	TAGACAAACA	CACACACACC	AGATAATATA	AACTTAATAA	ATTATCTGTT
	101	AATGTAGATT	TTATTTAAAA	AACTATATT	GAACATTGGT	CTTTCTTGA
	151	C				

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TCU9/1(2)

15	1	ACATAACAGC	TTTTATACAA	TGATAAGGAC	ATATCATTG	TTTACAAAGA
	51	AAGTCTAAAA	TTTCAAGAAC	ATTCAAAGAG	CTAACACAGT	AAAGGTCTG
	101	CAAGTTCTAG	AATAGTGAAT	CATGACAGAA	CTCATTCAATT	TTATCCTTAA
	151	TCTCC				

TCU9/2(2)

20	1	AAGTATGGGT	AGCTAAATT	GCATTAATT	AAAAGTACAT	ATAATGCAAC
	51	ACCACTCTAC	ATCTGTATAC	CTACGAATGT	ATGTGTACTA	CACACCCCTTA
	101	AAATGTTTT	CAAAGTCTTA	ATATATTAGA	ACATGTTTC	ATTTTTTCAT
	151	GGGATGTTAA	TACTATTCTA	TGATTAAGAA	AATACTAG	

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TCU10(2)

	1	AATACAGTTA	TTCTAGCTTT	TCATATTCAA	TTTGAATGAT	CAGAAAAGTA
	51	TATTAGTCAC	ACAGAATTAA	ATATTTAGA	TAGTAAGAAAT	C

30

TCU14(2)

	1	GAAGTGAAAG	TCAGCCCTTT	AGCTATTATT	TATTGCTTTA	TTAGAGCAGA
	51	GGGAAGTGAC	ACTCATTGCC	TTCACAGAGC	TCTGCAGAAA	TATATGCACA
	101	GAGTGGTCAA	TGCCAACATC	TGAGTAAGTC	TTCCAAA	

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TGO20(2)

40	1	CAGAACATTA	GGATTTATTC	CTTGATTAGT	TCAAATGATT	TCAACAGCTG
	51	AATTCCCTGA	GATGTGTAAG	GCAGGTTGGT	CCTTGGATG	GAATGTAGAC
	101	TGAAACTTCC	TATAACTGTA	GTGATATGTA	CACAGCTACA	TAGCAAAGTG
	151	CTTCATTATG	AAAATGAAGA	A		

TGO20(1)

45	1	CAGTGTGAGA	GTCTCATTTC	TATGCACAGT	GTTCCTCAGG	AGGATGGAGC
	51	TAGTTAGCTG	TCTGTTGTCT	GTAGCCCAGC	TTGATAATGG	AACTATACAG
	101	CGAAGAGACA	ATCTCTGGCA	AGTTTTGTA	GAA	

TGUS5(C)

50	1	TTAGAGTAA	ATTCCAATA	AATGCTTGC	TCCAAAATTA	CACTAACCAAG
	51	GCTGGGTCTC	TATCATAACAT	CTTCAATACC	CTCAAACCTA	GATTGTAAG
	101	TGAAAAAAAGT	GATTAGCNNT	TCCATTGTT	CATTCTGTCA	CTCACATTCT
	151	TAGGCATTTT	AAGGATGAGC	AACCTTTGTT	TCAGAAAGGG	TAAGTAATTA
	201	GCCCCCTGGA	GGTTACATAG	TTATAATTAA	GTCTTCAGAA	TCCGTTCGAA

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251	GGGNNNNNGTT	ACTATTTTA	AGATAATTAG	AACCCACCTT	GTAGCAATAA
301	AAGTTTTCTT	GTCTTTG			
5					
	TGU8(2)				
1	GCGAAAGACT	AATCGAACCA	TCTAGTAGCT	GGTTCCCTCC	GAAGTTCCCC
51	TCAGGATA				
10					
	TGU9/1(2)				
1	TTAATGTTTA	AATACTACTT	TTTTTCAAG	CTTGCCTAG	ATACCAACTG
51	TTTATCTAAC	ACACAATTCC	AGTGTGCGCA	AGCCTCATGC	CAATTGAAG
15	101	GGAACAGCCA	AAACTTATGC	ATTCATATAA	AAAGAGTCTC
151	TATCTACATT	ATAATTTT			TAGGCTCTTA
20					
	TGU9/2(2)				
1	GGAATAACAT	TTTTTTATGA	GGGAACCCCTT	TAAAATGGAT	GCACACAGTG
51	GCATTTCTC	CTAGGCTCAA	AGCTGAGTAC	ACTCCCGTAA	TTTTAATAAT
101	ATTTTAGGCA	AGTCCTATGA	CAATTATAACC	AAACAAGTTTC	TTCAACCCCCA
151	CCACCACCCC	ACCATCTCTA	TGC		
25					
	TGU12(C)				
1	GGAGGAAGCT	TTATTTGGGA	AGAGTGCCTG	TCNNTCGGCC	CTGATCAGCT
51	CTAGCCTGCC	CACCCCATCT	CAGCCAGGCG	GCTTTACTTC	TTCCCTGAGCT
101	TCAGGTCTT	CTTCTTCCTG	ATTCCCTTGG	CCAGCTCCCC	AATCAATCTC
30	151	CAGTACTCAT	TGAACCTTGAG	CTCCGAGNCC	TGATTACACAT
201	CATCTTCT				CCAAGCTCTT
35					
	TGU13/1(C)				
1	GGATGTGGTA	GTTGATCTTT	AATGCCATT	CTAGGTCCGA	AAAATCCATG
51	ATCCTAACTT	TTAAGAGAAAG	GTTGGTAACT	CTACTTAGGA	CTTTTTTTTG
101	TAAGAGGAAT	AATGTAGCCT	CACCCCTTATC	TTTCTGGAAA	TGTTTAAACC
151	ACTGAAATAT	GGAGATCAAA	TCCAGCTTAC	ACACTGGTAA	CTCAAATACT
40	201	ATTTTTTTT	TAAACTATCT	TTTCTAAACT	AATCACCCCT
251	GAACCTTCTA	TCTCAGTGCC	AATTCTTAGA	GGTTGATGCA	CTTGTACATA
301	CAGAGAGCCT	GTGCTATTGT	TC		AACAGCTCTC
45					
	TGU13/2(2)				
1	GGGGTGTACA	TTTTATTGGA	AACCTTAAAT	ACTGTTCAGA	AAGAATATAT
51	CTTCAATCAA	GGTCTTGCCG	AGCCTACACA	AAAAAATGAA	GCTTTTTGGG
101	TTAGGGCAA	GGATATATAC	AGTACAGAGG	ACAAAGA	
50					
	TTO16/2(C)				
1	ACATTCATTA	AAGATGAACT	TTCAGCATCT	TCACCTGAAG	ATCCATCAGA
51	TGATTCTGAG	AGGCAGGTCT	CCCCAAAAAA	TCCACCGCAT	GTATTCTTTC
101	GTTTAGAATC	TGAAAGCCTC	TTTCTTTCA	GGCTTGATGA	CTCTTCTAAG
151	GTATTTGTTA	TGCCTCTCTT	CTGGGTTTT	CGTTTGCCCT	TATCAAGTAG

	201	CTNAAATTCA	AACACCATGG	CAANAGAAAC	TGCTTCTAT
5		TTO20/1(C)			
	1	CCACCAGCCT	ACTGATCAGC	TGGGATGCTC	CTGCTGTCAC
	51	TACAGGATCA	CTTACGGAGA	AACAGGAGGA	AATAGCCCTG
	101	CACTGTGCCT	GGGAGCAAGT	CTACAGCTAC	CATCAGCGGC
10	151	GAGTTGATTA	TACCATCACT	GTGTATGCTG	TCACTGGCCG
	201	CCCGCAAGCA	GCAAGCCAAT	TTCCATTAAAT	TACCGAACAG
	251	ACCATCCCAG	ATGCAAGTGA	CCGATGTTCA	ACACAACGT
15	301	GATTTACATT	CCAC		TTTAATAAAA
		TTO20/2(2)			
	1	TTGGTACAC	AGTCACACAA	CTGGGGGTCA	TTTCTAGAT
	51	GAACAAGTTC	TCTTCCAACA	AAGAAATGTA	CTGTAGAAAT
20	101	CATGAATTTC	ATATATTGTG	TACAAATATA	AGGTATGTAT
	151	AG			CTGAATACAA
		TTU2/1(2)			
25	1	CTAGAACTTC	CAAAGGCTGC	TTGTCATAGA	AGCCATTGCA
	51	AACGGCTCCT	GTTAAATGGT	ATCTCCTTC	TGAGGCTCCT
	101	ATTTGTTACC	TAACCTTAT	GTGCCTTAAC	AGGCCAATGC
		TTU 2/2(C)			
30	1	AACCAAGTATT	TCAAAACTAT	TATCTGGATT	CAAGATTAGT
	51	TGTTTTCTTA	TCAGTAAAAT	AGGTCTTCAG	ATCTGCATCT
	101	CATGTTTTTC	TTCATAGATA	CCCGTTTGG	GGTTTTGCG
35	151	AAAGTGCAGTT	TATAGTCCTC	TCCACATTAA	TCTG
		TTU3(1)			
	1	GGGTAGAAAG	CTGAATAATT	TATGAAGGAG	AGGGGTCAGG
	51	GAGGACCTAT	TGGTGCAGGGG	GCTTTGTATG	ATTATGGCG
40	101	GTAGTTACTG	GTTGAACATT	GTTTGTGGT	GTATATATTG
	151	TGCTCGGGGG	AATAAGTTAT	GTGATTAGGA	GTAGGGTTAG
	201	AAG			GATGAGTGG
		TTU 5/1(2)			
45	1	GACAAAAAAA	AAAAAACAGG	TTTTAAAGCT	AGAAATGAAA
	51	GTATCTAA	GGATAAGTTA	CTTTATTATA	CACTAGAAAC
	101	GCTGAAACT	TAAAAATCT	CACACTGCTG	AATGTCTCTG
50		TTU5/2(2)			
	1	GCATCCATTG	TACATTGTTT	GGTTTGAGGT	TACCATGAGG
	51	CTATCTTATA	ATTATTATT	TCAACCTGAT	AAAACCTAAC
	101	TAAACAAACA	AACGAAAA		ACTATTGCA

TTU9/1(1)

5 1 TAAAATACTG GTTCTTTAT TCTGCAATAT TTTAAAAAT CACATTTCA
 51 GCCAGGCGCA GTTTCCACA CCTGTAATCC GGCACTTTGG GAGGCTGAGA
 101 TGGGTGGATC ACAAGGTAGG AGATCAAACA TCCTGGCAA CATGGTGAAC
 151 CTGTTTACT

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TTU9/2(2)

15 1 CAAGTATGGG TAGCTAAATT TGCATTAAT TAAAAGTACA TATAATGCAA
 51 CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCCTT
 101 AAATGTTCA AAGCTTAATA TATTAGAACCA TGTTTCATT TTCAGGGAG

20 TTU13(2)

1 GGAAATACAC TAGCATGTGA GCACGTATA TAAAGCTTGA GGTTAGGAGG
 51 TAAAATGAAA GAAATCATT TTAACTCCTA AGATGT

25 TTU13(1)

1 TGAATTAAAT GGACTCGTTG AAAGGACAAG GAGATCGGTA ATATCTCTCT
 51 AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT
 101 CTTCTTTT

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or an analog thereof. In accordance with the invention, the term "analog" includes nucleic acids which code for the same protein sequence due to the degeneration of the genetic code, for a protein having conservative amino acids substitutions or deletions that do not eliminate the characteristical feature of this protein, or for a protein having at least about 85 %, and more advantageously at least about 90 %, in particular 95 % amino acid sequence homology.

35 Other embodiments of the invention provide a vector containing said DNA and a host cell containing said vector.

According to the general knowledge one skilled in the art can also use said nucleic acids of the present invention as a hybridization probe to detect the corresponding genes in an organism or in a sample from on organism or gene 40 mutations thereof.

Therefore, an additional embodiment is a method for isolating a gene which can be induced or repressed by treating chondrocytes that contain this gene by IL-1 β containing the steps:

45 (a) hybridizing a DNA of the present invention under stringent preferably high stringent conditions against DNA or RNA containing said gene, preferably DNA or RNA isolated originally from chondrocytes, in particular human chondrocytes; and

50 (b) isolating this gene by methods known to a skilled person in the art.

According to the present invention the term "stringent conditions" means hybridization conditions comprising a salt concentration of 4 x SSC (NaCl-citrate buffer) at 62-66°C, and "high stringent conditions" means hybridization conditions comprising a salt concentration of 0,1 x SSC at 68°C. The length of the probes are 6-100, preferably 10-40, in particular 12-25 nucleic acids long.

55 Yet another embodiment is a process for expressing a gene isolated according to the above-described process containing the steps:

(a) cloning said gene into a suitable expression vector such as the pET series (Studier et al., 1990. Methods in Enzymology 185, 60) for procaryotic expression or the vector CDM8 for mammalian expression (Aruffo and Seed, 1987. Proc. Natl. Acad. Sci. USA 84, 8573) or any other expression system known to one skilled in the art; and

(b) expressing said gene in a suitable host cell such as BL21 series (Studier et al., 1990, *supra*) for prokaryotic expression or COS, cells for mammalian expression (Aruffo and Seed, 1987, *supra*) or any other expression system known to one skilled in the art;

5 or a method for producing a protein containing the steps:

(a) culturing a suitable host cell, in particular the above mentioned, containing a vector, in particular an expression vector such as the vectors mentioned above which contains a DNA or a gene of the present invention; and

10 (b) isolating the expressed protein for example by ultrafiltration, precipitation with chaotropic agents such as urea or column chromatography on e.g. ion exchange chromatography columns as detailed in Ausubel et al. 1994 (*supra*).

A further embodiment is a diagnostic aid containing a DNA or parts thereof or a gene or parts thereof of the present invention. In particular, quantification of the genes can be achieved on the RNA level by Northern blotting with gene 15 specific probes of the present invention or with gene specific primers in a PCR reaction. Such primers can be synthetically produced using the DNA sequences of the present invention or the sequences of the corresponding genes. Therefore, said nucleic acids are useful for the diagnosis of IL-1 β related diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

These nucleic acids can also be used to evaluate the expression of certain genes in small cartilage biopsies and 20 to use these ultimately as disease-specific markers and/or as predictive markers for disease progression of e.g. osteoarthritis. The hybridization conditions can be the same as described above.

Said nucleic acids, however, can also be used for the therapy against the diseases mentioned or for the production of a pharmaceutical.

Therefore, another embodiment of the present invention is also the use of said nucleic acids for the production of 25 a pharmaceutical. For example, as described by Uhlmann & Peyman (Chem. Rev. (1990), 90, 543), Milligan et al. (J. Med. Chem. (1993), 36, 1923) or Stein & Cheng (Science (1993), 261, 1004) such nucleic acids can be used as antisense oligonucleotides or triple helix forming oligonucleotides for the inhibition of gene expression. This is in particular useful if such a disease is caused by the overproduction of a gene product which is directly or indirectly regulated by IL-1 β in 30 chondrocytes. The nucleic acids can additionally be modified in order to increase e.g. the stability against nucleases as described e.g. in the literatures mentioned above.

Finally, also the gene product itself produced by a method of the present invention can be used as a pharmaceutical. In the following the invention is in particular described by the examples and tables:

Description of the Tables

35 Table 1 gives an overview on used primers and the complexity of the detected differences in expression. Table 2 summarizes the result of the sequencing of differentially displayed PCR products after their elution from the sequencing gel, reamplification and subcloning into the pCRII vector. The sequences of TAU1/1(1) and TAU1/1(2) are 100 % identical to human osteopontin cDNA, the sequence of TTU2/2 is 97.2 % identical to human calnexin. bp = base pairs, IL-1 = Interleukin-1 stimulation, Stat. sig. score = statistical significance score: a feature of the BLAST database 40 searching program. This score is determined using an implementation of Karlin's significance formula (Karlin, S. and Altschul, S.F. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc. Natl. Acad. Sci. USA, 87:2264-2268), which calculates the Poisson probability that the observed sequence similarity will occur by chance based on the size and composition of the sequence database as well as on 45 the size and quality of the match. The smaller this number, the more it is likely to see sequence similarities.

Examples

Cell culture

50 Articular cartilage specimen were obtained from two patients (male 65 years old and female 73 years old) undergoing total joint replacement surgery for osteoarthritis. None of these individuals had received treatment by radiation or chemotherapy. Articular cartilage slices were aseptically dissected from both femoral condyles, tibia plateaus and patellae and subjected to sequential enzymatic digestion with pronase and collagenase as described (Häuselmann HJ et al. 55 1992, Matrix 12, 116-129) Since it is known that the alginate gel suspension system retains the chondrogenic phenotype [Lohmander LS et al. 1992, Trans. Orthop. Res. Soc. 17, 273.] 4 x 10⁶ chondrocytes were suspended in low viscosity alginate (4 x 10⁶ cells / ml 1,25 % w/v alginate in an isotonic buffered solution) and expressed through a 22gauche needle into 102 mM CaCl solution to form cell entrapping beads which are 1,5-3 mm in diameter and spherical. Alginate beads containing a total number of 2 x 10⁷ cells were fed daily for the first three days with medium F12 / DMEM (50/50)

and 10 % fetal calf serum (Sigma) with 25 µg / ml ascorbate and 50 µg / ml gentamycin and were then subdivided into two populations for further three culture days in the presence or absence of 5U / ml rh IL-1 β (Genzyme). For cell recovery, alginate beads were finally dissolved into dissolution buffer (55 mM sodiumcitrate, 30 mM EDTA, 0,15 M NACl) and placed at room temperature for 10 min. Viability was checked by eosin-red exclusion and cell number was determined.

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Primer syntheses

Arbitrary oligodecamer primers OPA6 to OPA10, OPA16 to OPA20 and degenerate anchored oligo-dT primers (T₁₂VN) were synthesized using the 392 DNA synthesizer (Applied Biosystems) and purified by denaturing polyacrylamid 10 gel electrophoresis. Some oligodecamer primers, U1 to U15 were purchased from Biometra (Göttingen, FRG).

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5 List of all degenerate 3' oligo dT-primers [T₁₂VN] used for DDRT-PCR:

Primer	Sequence 5' to 3'
T ₁₂ VA	5'-TTTTTTTTTTTTVA-3'
T ₁₂ VA	5'-TTTTTTTTTTTTVT-3'
T ₁₂ VA	5'-TATTTTTTTTTVG-3'
T ₁₂ VA	5'-TTTTTTTTTTTC-3'
V = dA, dG, dC; N = dA, dT, dG, dC	

10 List of all arbitrary 5' oligodecamer primers used for DDRT-PCR:

Primer	Sequence 5' to 3'
OPA 6	GGTCCCTGAC
OPA 7	GAAACGGGTG
OPA 8	GTGACGGGTG
OPA 9	GCGTAACGCC
OPA 10	GTGATCGCAG
OPA 16	AGCCAGCGAA
OPA 17	GACCGCTTGT
OPA 18	AGGTGACCGT
OPA 19	CAAACGTCGG
OPA 20	TTTGCATCC
U1	TACAACGAGG
U2	TGGATTGGTC
U3	CTTCTACCC
U4	TTTGGCTCC
U5	GGAACCAATC
U6	AAACTCCGTC
U7	TCGATACAGG
U8	TGGTAAAGGG
U9	TCGGTCATAG
U10	GGTACTAAGG
U11	TACCTAAGCG
U12	CTGCTTGATG
U13	GTTCAGCGAG
U14	GATCAAGTCC
U15	GATCCAGTAC

RNA isolation and cDNA synthesis

5 Total RNA from cultured articular chondrocytes was prepared according to a single step method Chomczynski and Sacchi (Chomczynski P & Sacchi N 1987, Anal. Biochem. 162, 156-159) and incubated with 10 U RNasefree DNaseI (Gibco, Eggenstein, FRG) for 30 min at 37°C to remove chromosomal DNA contamination of RNA. After extraction with phenol/choroform (3:1), the supernatant was ethanol precipitated in the presence of 0.3 M NaOAc and RNA was redissolved in DEPC treated water. 0,4 µg total RNA was then reverse transcribed using 200 U M-MLV (Moloney murine leukemia virus) reverse transcriptase (Gibco, Eggenstein, FRG) in a 40 µl reaction volume containing 50 mM Tris-HCl (pH 8,3), 75 mM KCl, 10 mM DTT, 3 mM MgCl₂, dNTP mix (dATP, dTTP, dCTP, dGTP) of 200 µM each, 40 U RNase inhibitor (Boehringer Mannheim, FRG) and 2,5 mM degenerate oligo-dT primer (T₁₂VN) at 37°C for 1 h. Reactions were 10 terminated by heating for 5 min at 95°C.

PCR amplification

15 20 cDNAs were amplified in a DNA thermal cycler (Perkin Elmer, model 480) in 20 µl PCR reactions containing 2.5 µM of the original T₁₂MN-primer used in cDNA synthesis in combination with 0.5 µM arbitrary upstream primer, dNTP mix (dGTP, dCTP, dTTP) of 0,5 µM each, 10 µCi α-[³⁵S]dATP (1000 Ci/mmol, 10 mCi/ml), 10 mM Tris-HCl (pH 8.3) 50 mM KCl, 1,5 mM MgCl₂, 0,001 % gelatin and 2,5 U AmpliTaq DNA polymerase. Light mineral oil was overlaid and thermal cycling was performed as follows: 94°C for 30 seconds, 40°C for 2 min and 72°C for 30 seconds for 40 cycles followed by 5 min postextension at 72°C. AmliTaq DNA polymerase was purchased from Perkin-Elmer (Weiterstadt, FRG) and α-[³⁵S]dATP was obtained from Amersham-Buchler (Braunschweig, FRG). After addition of 5 µl stop buffer (95 % formamide, 20 mM EDTA, 0,05 % bromphenolblue and 0,05 % xylene cyanol) radiolabeled PCR-fragments were then displayed on 6 % acrylamide/7 M Urea high resolution sequencing gels of 35 x 43 cm in size; dried gels were exposed to X-ray film (Kodak X-OMAT) and exposed for 48 h, which allows rapid identification of differentially expressed genes 25 by side by side comparison of DDRT-PCR band patterns.

Elution, reamplification and cloning of PCR fragments

30 35 PCR fragments identified as differentially expressed bands were cut from acrylamide gels, transferred into Eppendorf tubes and rehydrated for 10 min with 100 µl 10 mM Tris-HCl and 1 mM EDTA at room temperature. After boiling the gel slice for 15 min, the PCR fragment was recovered by ethanol precipitation in the presence of 0.3 M NaAc and 20 µg glycogen as a carrier and redissolved in 10 µl sterile water. 5 µl of this volume was used for reamplification by PCR using appropriate primers and conditions described above except for dNTP concentration of 20 µM and no radioisotope. The reamplified PCR product was visualized by electrophoresis on a 2 % agarose gel and eluted from the gel by ultrafiltration 40 using Ultrafree MC-filters (Millipore). Purified PCR fragments were then cloned into the pCRII-vector (Invitrogen, De Schelp, NL) by the TA cloning method (Kovalic D et al. 1991, Nucleic Acids Research 19, 4640), which allows in-vitro transcription and sequencing from the plasmid.

Sequencing

40 Plasmid DNA sequencing of subcloned PCR fragments with either SP6(2) or T7(1) primer was carried out using the chain-termination DNA sequencing method, as described by Sanger et al. (Sanger F et al. 1977, Proc. Natl. Acad. Sci. USA 74, 5463-5467.).

45 Sequence analysis

The sequence analysis revealed the sequences of cDNA clones TAO8/2(2), TAO16/1(2), TAO16/2(2), TAO17(c), TAO19(c), TAU1/1(2), TAU1/1(1), TAU1/2(2), TAU7/1(2), TAU7/1(1), TAU7/2(c), TAU10(1), TAU12/1(2), TAU12/1(1), TAU12/2(1), TAU12/3(2), TAU12/3(1), TAU13/1(1), TAU13/3(2), TAU13/3(1), TCO16/1(c), TCO16/2(c), TCO17(c), TCO18(c), TCU2/1(1), TCU2/2(1), TCU9/1(2), TCU9/2(2), TCU10(2), TCU14(1), TCU14(2), TGO20(2), TGO20(1), TGU5(c), TGU8(2), TGU9/1(2), TGU9/2(2), TGU12(c), TGU13/1(c), TGU13/2(2), TTO16/2(c), TTO20/1(c), TTO20/2(2), TTU2/1(2), TTU2/2(c), TTU3(1), TTU5/1(2), TTU5/2(2), TTU9/1(1), TTU9/2(2), TTU13(2), TTU13(1) disclosed on pages 7 to 14 of the specification.

50 55 Searching for homology between subcloned PCR fragments and sequences already listed in one of the DNA databases (GenBank or EMBL database) was performed using the FASTA program developed by Pearson and Lipman (Pearson W & Lipman DJ 1988, Proc. Natl. Acad. Sci. USA 85, 2444-2448) included in the GCG software package (Genetics Computer Group, Madison, USA).

Northern-blot analysis

Cell culture and isolation of RNA was performed exactly as described above. 10 µg of total RNA from both IL-1 β stimulated or not stimulated chondrocytes were denatured by heating at 65°C for 10 min in a solution of 50 % formamide, 5 20 mM MOPS and 2.2 M formaldehyde, separated through a 1 % agarose gel containing 2.2 M formaldehyde in 1 X MOPS and transferred to positively charged nylon membrane (Amersham) by standard blotting procedures [Maniatis et. al 1992]. After UV crosslinking, the blots were prehybridized for 1 h in rapid-hyb-buffer (Amersham) at 65°C. A 330 bp cDNA corresponding to nts 61 to 390 of human osteopontin cDNA (GenBank J04765) and a 340 bp cDNA corresponding to nts 881 to 1220 from human calnexin (GenBank M94859) were radiolabeled for hybridization with α -[³²P]dCTP (3000 10 Ci/mmol, 10 mCi/ml) using random nonamer primers (Amersham) up to a specific activity of $\sim 1,5 \times 10^9$ dpm / µg DNA. Hybridization was performed for 2,5 h at 65°C in prehybridization solution with 2 ng / ml of labeled probe added. The blot was subsequently washed in 2 X SSC, 0.1 % SDS at 37°C for 15 min (1 X SSC = 0,15 M NaCl, 0,015 M sodium citrate, pH 7,0), followed by two successive washes with 1 X SSC, 0.1 % SDS at 65°C for 10 min respectively. If necessary, a final high stringency wash was performed with 0.1 X SSC, 0.1 % SDS at 65°C for 15 min. The blots were then analysed 15 by autoradiography using Kodak X-Omat films at -80°C with intensifying screens for 2-7 days and intensity of bands was quantified with a phosphorimager (Biorad, model GS-250). All blots were stripped with boiling 0.5 % SDS solution and reprobed with labeled β -actin to demonstrate equal loading of RNA in each lane.

Northern hybridisations (Results)

20 Fragment TAU7/2(c), identical to TSG-6, was differentially upregulated in IL-1 stimulated cells. This is in concordance with Lee et al. (1992) which reported for TSG-6 a TNF- α and IL-1 mediated upregulation. Fragment TAU1/1, identical to human osteopontin and fragment TTU2/2, identical to human calnexin, both were weaker expressed in IL-1 stimulated chondrocytes compared with the unstimulated cells. To validate our differential display data, we performed Northern 25 analyses of Osteopontin and calnexin expression in IL-1 stimulated and unstimulated chondrocytes originating from a third patient. Both messages were again downregulated. A phosphorimager quantification revealed an osteopontin downregulation by 79% and a calnexin downregulation by 40% in the RNA population from chondrocytes of the third

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Table 1: Current results of differential display reverse transcriptase PCR (DDRT-PCR) to reveal differential gene expression by chondrocytes with and without IL-1 β

Overview on used primers and number of analysed bands

DDRT-PCR primercombination	5'-Oligodecamer (downstreamprimer)	putative differential expressed genes by side by side comparison	reproducibility of DDRT-PCR band pattern from first to second, third or reamplified in PCR	eluted from gel and sequenced using SP6	cloned into pCRII vector by TA cloning	method verified by PCR or T7 promoter
T ₁₂ M [*] A	OPA 6 - OPA 10	25 out of ~ 4000 bands	7 not done	6	1	
T ₁₂ M [*] T	OPA 16 - OPA 20	19 out of ~ 4000 bands	13 9	12	12	12
T ₁₂ M [*] G	U 1 - U 5	31 out of ~ 4000 bands	not done	11	10	10
T ₁₂ M [*] C	U 6 - U 10	27 out of ~ 4000 bands	not done	13	11	11
	U 11 - U 15	21 out of ~ 4000 bands	not done	11	10	10
total 4 x	25	total 123	total	55	total 52	total 44
	= 100 combinations					

* means threefold degeneracy where M may be dA, dG or dC

1 patient female 73 years old diagnosis gonarthrosis

2 patient male between 65-75 years old

theoretical consideration:

Suggesting that an arbitrary upstream primer detects 3 % of the total RNAs (Liang 1994), then 97 % of the total mRNAs will not be detected, i.e. with 25 arbitrary oligodecamerprimer and the four degenerate T₁₂VN primers, about half of the mRNAs would be seen ($P = 1 - (0.97)^{25} = 53.3\%$) in 100 lanes of high resolution sequencing gels.

Table 2 IL-1 mediated differentially displayed cDNA fragments of human articular chondrocytes

Fragment	bp	IL-1	Features	Stat.sig.score
TAO 8/2(2)	275 bp	+	146 bp sequenced, no homology found	0.999
TAO 16/1(2)	450 bp	+	80 bp sequenced, no homology found	0.69
TAO 16/2(2)	200 bp	+	115 bp sequenced, no homology found	0.04
TAO 17(c)	412 bp	+	412 bp sequenced, no homology found	0.016
TAO 19(c)	209 bp	--	209 bp sequenced, no homology found	0.99
TAU 1/1(1,2)	450 bp	--	100 % sequence identity to human osteopontin cDNA in 303 bp overlap (303 bp seq.)	1.2×10^{-101}
TAU 1/2(2)	430 bp	+	188 bp sequenced, no homology found	0.82
TAU 7/1(1,2)	500 bp	+	87 % sequence identity to human cDNA clone c-1sd02 in 125 bp overlap (235 bp seq.)	8.1×10^{-33}
TAU7/2(c)	202 bp	+	99.5 % sequence id to human TNF stimulated gene-6 in 202 bp overlap	4.8×10^{-76}
TAU 10(1)	400 bp	+	181 bp sequenced, no homology found	0.9997
TAU 12/1(1,2)	470 bp	--	319 bp sequenced, no homology found	3.3×10^{-14}
TAU 12/2(1)	390 bp	--	155 bp sequenced, no homology found	0.0078
TAU 12/3(1,2)	250 bp	--	95 % sequence identity to human cDNA clone HRBBA21 similar to S10 in 158 bp overlap (162 bp seq.)	1.0×10^{-28}
TAU 13/1(1)	600 bp	+	145 bp sequenced, no homology found	0.12
TAU 13/3(1,2)	500 bp	--	439 bp sequenced, no homology found	0.33
TCO 16/1(c)	241 bp	+	241 bp sequenced, no homology found	2.4×10^{-7}
TCO 16/2(c)	230 bp	+	230 bp sequenced, no homology found	4.3×10^{-5}
TCO 17(c)	169 bp	+	169 bp sequenced, no homology found	0.49
TCO 18(c)	168 bp	+	168 bp sequenced, no homology found	1.3×10^{-6}
TCU 2/1(1)	400 bp	+	178 bp sequenced, no homology found	0.66
TCU 2/2(1)	210 bp	+	151 bp sequenced, no homology found	0.0074
TCU 9/1(2)	430 bp	+	99 % sequence identity to human cDNA clone 131036 3' in 155 bp overlap (155 bp seq.)	7.2×10^{-58}
TCU 9/2(2)	320 bp	--	188 bp sequenced, no homology found	0.22
TCU 10(2)	320 bp	--	100 % sequence identity to human cDNA clone 26518 3' in 85 bp overlap (91 bp seq.)	2.9×10^{-28}

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Fragment	bp	IL-1	Features	Stat.sig.score
TCU 14(1,2)	280 bp	+	99.3 % sequence identity to human cDNA HL60 3'directed MboI in 249 bp overlap (249 bp seq.)	3.5×10^{-51}
TGO 20(1,2)	300 bp	+	304 bp sequenced, no homology found	0.95
TGU 5(c)	317 bp	+	317 bp sequenced, no homology found	0.088
TGU 8(2)	320 bp	+	100 % sequence identity to human 28S rRNA in 58 bp overlap (58 bp seq.)	1.4×10^{-16}
TGU 9/1(2)	280 bp	+	169 bp sequenced, no homology found	0,55
TGU 9/2(2)	220 bp	--	100 % sequence identity to human cDNA clone 12A10B in 100 bp overlap (173 bp seq.)	4.0×10^{-30}
TGU 12(c)	208 bp	--	87 % sequence identity to human cDNA clone 113442 3' in 208 bp overlap	5.5×10^{-83}
TGU 13/1(c)	322 bp	+	322 bp sequenced, no homology found	6.9×10^{-13}
TGU 13/2(2)	300 bp	--	94.9 % sequence identity to human F1 ATPase β -subunit in 137 bp overlap (137 bp seq.)	2.3×10^{-43}
TTO 16/2(c)	239 bp	+	97.5 % sequence identity to human ERCC5 in 239 bp overlap (239 bp seq.)	9.3×10^{-88}
TTO 20/1(c)	314 bp	+	100 % sequence identity to human fibronectin in 314bp overlap (314 bp seq.)	1.9×10^{-121}
TTO 20/2(2)	400 bp	+	152 bp sequenced, no homology found	0.035
TTU 2/1(2)	300 bp	--	100 % sequence identity to human cDNA clone 118470 5' in 146 bp overlap (146 bp seq.)	2.1×10^{-30}
TTU 2/2(c)	184 bp	--	99 % sequence identity to human calnexin in 184 bp overlap (184 bp seq.)	2.3×10^{-84}
TTU3(1)	400 bp	+	97 % sequence identity to human NADH-DH mtDNA subunit in 203 bp overlap (203 bp seq.)	8.6×10^{-89}
TTU 5/1(2)	300 bp	--	147 bp sequenced, no homology found	0.0065
TTU 5/2(2)	270 bp	--	118 bp sequenced, no homology found	0,035

Fragment	bp	IL-1	Features	Stat.sig.score
TTU 9/1(1)	350 bp	+	94 % sequence identity to human cDNA clone 83764 3' in 159 bp overlap (159 bp seq.)	5,9 x 10 ⁻²³
TTU 9/2(2)	320 bp	--	149 bp sequenced, no homology found	0,22
TTU 13(1,2)	350 bp	+	194 bp sequenced, no homology found	0,57

Thus, the 44 identified fragments can be subdivided as follows:

1) 2 fragments with sequence homologies to known human genes with known roles in IL-1 mediated processes:

TAU 7/2 identical with human TNF-stimulated gene-6
 TTO 20/1 identical with human fibronectin

2) 6 fragments with sequence homologies to known human genes, whose function in IL-1 mediated processes can be speculated:

TAU 1/1 identical with human osteopontin
 TGU 8 identical with human 28S ribosomal RNA gene
 TGU 13/2 identical with human F1 ATPase β -subunit
 TTO 16/2 identical with human ERCC5
 TTU 2/2 identical with human calnexin
 TTU 3 identical with human NADH-DH mtDNA subunit

3) 9 fragments with sequence homologies to human genes, identified in human genome sequencing projects:

TAU 7/1 identical with human cDNA clone c-1sd02
 TAU 12/3 identical with human cDNA clone HRBBA21
 TCU 9/1 identical with human cDNA clone 131036 3'
 TCU 10 identical with human cDNA clone 26518 3'
 TCU 14 identical with human cDNA clone HL60 3' directed MboI
 TGU 9/2 identical with human cDNA clone 12A10B
 TGU 12 identical with human cDNA clone 113442 3'
 TTU 2/1 identical with human cDNA clone 118470 5'
 TTU 9/1 identical with human cDNA clone 83764 3'

4) 27 fragments without sequence homologies to known human genes. The detection of TSG-6 and fibronectin, both genes known to be upregulated by IL-1, points to the importance of those other cDNA fragments in the light of IL-1 mediated processes. Those genes very likely play roles in degenerate joint diseases, including rheumatoid and osteoarthritis and with this are interesting candidates as markers for clinical studies or as drug targets for pharmaceutical intervention.

Claims

1. Use of osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis.
2. Diagnostic aid for the diagnosis of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

3. Pharmaceutical for the prophylaxis or therapy of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.
5. 4. Use of calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis.
10. 5. Diagnostic aid for the diagnosis of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.
15. 6. Pharmaceutical for the prophylaxis or therapy of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.
20. 7. Use of TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis.
25. 8. Diagnostic aid for the diagnosis of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.
9. Pharmaceutical for the prophylaxis or therapy of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

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10. DNA containing a DNA selected from the group consisting of

TAO8/2(2)

5 1 CCAAGTTTT CCAGCAACCC CAAGGGAATA CAGGGAGATC AATGCACCCA
51 51 AAATGGGAAA AGAAAAATAC TTCGATGCAA TGAAACAAAG CCTTTTCCG
101 101 TTCAGTTCC ATAATTCACT GGTCAGTTT AAGGCTGCCA CTTGGG

10 TAO16/1(2)

1 1 GACACGAACA CCACATATTT TTATTGGAGG CCCCATGGCT CCTTGGAAAGC
51 51 CATTGGAA CCAAGGGAC CCACCTTTT

15 TAO16/2(2)

1 1 CTAAATATAT TCTCTAACAA GTTAATCTCT TTCAAATCTA TAGATAAAAC
51 51 TAAAAGGATA AGGAACCAAG GTTTAACCGA CCTAGCCAAT TATGGCAATC
101 101 ATACTTGCTT TTTAG

20

TAO17(C)

1 1 CATGAAATAT TTCTTGAGGT AATAAGCTT TACCAAGCTT ATATTTTGG
51 51 GCAATTCACT TACAATGAGA AAAAAACACA CCAAAAGACC AAAAATTAA
101 101 AAAACTCACT TTTCTTGCAA TCATAGACAT TTGCATTATT ATAGAACATT
151 151 CAAACAAGTT AGGTGGATAA TTATTGTCTA TAGATAAAATA CGATGCAATT
201 201 TTAATAAGAA TTTGAAGAAT GACATTAAT GCTGTCTGAA GCCTTTGTAT
251 251 TTTTTAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA
301 301 AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTAAC

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351 AGATATGATG ACAGNAAAAA TTGATTGCAT ATCTTTTGC ACTAAAAC
 401 TTATATTTAT TT

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TAO19 (C)

1 AGAGCAGGGG TATTCNCGG TTCATACCGC CATGGCTAA GAAGCAAAAG
 51 TCATATACCT TAGTAGTGGC AAAGATNGAG GAGATAAAA AGAGCCTACC
 101 CAAGCTGTTG TTGAAGAACCA CCTCTTAGAT AAAGAGGAAC CCTTCCAGAA
 151 GNACAGAGAC AGGCTAAGGG TGATGCTGAG GAAATGGCTC AGAAGAAACA
 201 AGAGATTAA

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TAU 1/1(2)

1 CTAATGCAA AGTGAGAAAT TGTATTTTT CTCCTTTAA TTGACCTCAG
 51 AAGATGCACT ATCTAATTCA TGAGAAATAC GAAATTCAG GTGTTATCT
 101 TCTTCCTTAC TTTGGGG

20

TAU 1/1(1)

1 ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC
 51 ATCCCCGTTT CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG
 101 GAAGGACAGT TATGAAACGA GTCAAGCTGGA TGACCAAGAGT GCTGAAACCC
 151 ACAGCCACAA GCAGTCCAGA TTATATAAGC GGAAA

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TAU1/2(2)

1 CCGGAATGGG GAGCAAACCA TAAGAACCGG GACCAGTTTC CTCTCTTGT
 51 GCCCTAGTTC CCCCTCCTT GTATACACCC TCCATCCTGA ATAGACTCTG
 101 GTTCTCAGCG TAACACCGAC AACATTCAAT CCTGTAGAGA AACAAATGTT
 151 AGCTCAGAAG GACACAGCCT TTGAATCATC AGAGAGTT

30

TAU 7/1(2)

1 GTTAAGAATA ACTAAATAAA AGTTTTAATT AATTTAGGAA TATAAAAAC
 51 TATTAACATT TAATTTATA ACTGTATCTG CCAAGCAACT TAAATATAA
 101 TTTATTTACC

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TAU 7/1(1)

1 CACGCAATGT GAAATAGGCA CATAGGAAGA ATGGGAAAC CATCCCTCA
 51 AGCATTATC CTTTGAGTTA CAAGCAATCC AATTACACTC TTTAGTTAT
 101 TTTAAATGT ACAGTTAGGT TATTA

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TAU 7/2(C)

1 CCTTGAAGAT GACCCAGGTT NCTTGGCTGA TTATGTTGAA ATATATGACA
 51 GTTACGATGA TGTCCATGGC TTTGTGGAA GATACTGTGG AGATGAGCTT
 101 CCAGATGACA TCATCAGTAC AGGAAATGTC ATGACCTTGA AGTTTCTAAG
 151 TGATGCTTCA GTGACAGCTG GAGGTTTCCA AATCAAATAT GTGCAATGG
 201 AT

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TAU10(1)

5 1 GGAGATGACA TTTGCTTTGG GCAGAGGCAG CTAGCCAGGA CACATTTCCA
 51 CTATAATTT ACAAAAGTTAA ATTTATAAGC TAGCATTAAAG TAAAGTGAAG
 101 TTCCAGCTCC CTTGCTAAAA ATAACCTAGAG GTAATAATTG GTATTCAGGT
 151 AACTCATTAA CATCATAATG TGTTGTGAAA A

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TAU12/1(2)

1 TATAAAATAT AAATTATATT ATAAATCATG TATTATTTAT AAAATTATAT
 51 TATAAAATTAA TAAAAATATA AATTATATT TAGGCTTAAT GTATAAGGAA
 101 TATAAAATTAT TAATAAGCAT ATGA

15

TAU 12/1(1)

20 1 TGTAATTAAC TGTNCTTGTA GGTCTGTCTT TTATACATGT GTGAGTTTT
 51 CTTTACAATA GATTCCCTAGC ATTGGGATTG CTAGGTCAGA TGGTATGCAC
 101 ATTTGACATT TTGATTGATA GCACCAGATT GCTTTGTTAA AAAATTTNN
 151 TTTATAGTTT ACATTATCTT TGTACAATAG ATGTTCTCTT TCGAC

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TAU 12/2(1)

25 1 GGGAAAGTGAA TTGAAAATAC TTCTTTNTCA ACATAATTTT NGGGTTTTGA
 51 AATTGTGTTT GGGTTTTCAG GAAATTGGTG GTAATCTTGT ATTAGCTGAA
 101 AAAAAGTGAA TTTAAAATT CTCAGTGAAG AAGCAAATGA TTTATTTTC
 151 ATAGA

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TAU12/3(2)

30 1 TGTTCTGGTA ACTGTTCTAA TTGTGTCTTT GTTACTTCCA GTGCAACCCCT
 51 TTCAGGTAAG

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TAU12/3(1)

40 1 CTAAGAACT TGGTATCTCT ATTAAAGCAC ACGAACCTCC AAGGAAAATA
 51 GAGCGATTTA CTCTTCTCAT ATCAGTGCAT ATTATATAAGA AGCACGGAGT
 101 CA

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TAU13/1(1)

45 1 AGTCATCAAT TCCCTTTTAT CTGTAATTAC ACATTTGTTT TTATTTCAA
 51 GTAATTATAA GGTGTTATAT TGCAATATAAT CAGAAAACCA AATGGAAATA
 101 AAATTTAGT AAGCCCGGCC CCTTTGACCG ATACAGAAAAA CTTGA

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TAU 13/3(2)

50 1 TATATGGCAG TCTAAAGCAT CAAAGATTG CATCAACATC TTTCATTAA
 51 GACATCTCCT TGCAATGTAA AATATCATGT ATCAACAACA TCTGGTGCAA
 101 ATCCCATGAGT CTAACTCGAC ATTCACTCTTA GCTCGATTAT TATTCCTTCG
 151 TACAGTCGAT GTAAACAATA CAGAAAGAGG ATTATTAAGA ACAGTTT

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TAU 13/3(1)

5 1 ATTCACTGAAA TGGTCTATAT GCATGATATT GTAAATTGGG ACTCGAAACC
 51 51 GAAACCAACG ATTCCGTTAC AAAAATTCCCT TAATGCTGAG AATGTTCTCA
 101 101 CGCAAACAAC ATCATGGACA TTAAATTCAA GATATGTGAA TGTAAATTCT
 151 151 GTCAATAAAG TCAACGTAAG GAGTAAAGTT AAAAACAGTT ATATCTNNNC
 201 201 TGTCAATGAT GAGTTAGTT TAACAGATGA TGAATCAATT CT

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TCO 16/1(C)

15 1 CAAAGTGTGTT TTGGTTTGA GAGAGAGAGA GATTGAGAGA CAGAGAGAGA
 51 51 GAGAGAAACC AAGGGATCAT GATAGTTATA GTCAAATACG AGGTTGGATT
 101 101 ATCTTTGAA AATGTGTTGG TTCTGTGATA CAAGAGGAAG CTAAGACATA
 151 151 TCGTGGAAAC ATCTCCCCCC TCCACCTTAA TATCAAGAAC AAATTGTGGA
 201 201 ATCTAATGTT AATGAGAAGT AGTTCCCCAC TGTGTCAGAT G

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TCO16/2(C)

20 1 NCATCTGACA CAGTGGGGAA CTACTTCTCA TTAACATTAG ATTCCACAAT
 51 51 TTNNNCTTGA TATTAAGGNN NNNNNNGGAG ATCGTTTCAC GATATCGTCT
 101 101 TAGCTTCCTC TTGTATCACA GAACCAACAC ATTTCAAAAG ATAATCCTTC
 151 151 CTCNNNTTGA CTATAACTAT CATGATCCCT TGGTTCTCTC TCTCTCTCTG
 201 201 CTCTCTCATC TCTCTCTCTC TNAAAACNAA

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TCO17(C)

30 1 ACAGTAGTTA GGAGTTCTT TACTTACAAA ATCACTGGAA ATGATTAAT
 51 51 TGCTTTTCCC CCTCCCCAGA GGTGCATTTT TCTTATTCC ATATAGTAAA
 101 101 GTTGAGCTTT TACAGTGCAT AATGTGACAT TTGGAATGCT TATCAACTGC
 151 151 ATGTAAACAT TAATAACCT

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TCO18(C)

35 1 GTAAATGGTA TTANNNGCTG AAGAAAAAAA ATTTTCAAG ACCTCTGTTT
 51 51 TTTAACTGAA CTTTATCATT GGCATTGTGG GCTTTGAAGT TGCTGGGATA
 101 101 AATTAATATA ATTAATAAA AGACTGAATT TAATTGCAA AAAAAAAA
 151 151 AACAAATAAGT GTGGTGAT

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TCU2/1(1)

45 1 AAGAAATTAT CCAGTTATTT ACAAGGCCAC TGATATTTA AACGTCCAAA
 51 51 AGTTTGTGTT AATGGGCTGT TACCGCTGAG AATGATGAGG ATGAGAATGA
 101 101 TGGTTGAAGG TTACATTTA GGAAATGAAG AAACTTAGAA AATTAATATA
 151 151 AAGACAGTGA TAAATACAAA GAAGATTT

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TCU2/2(1)

50 1 CGGGTTAATA TTATCCTCTA GTATAAGTGA ATTACTAGTT TCTCTTTATT
 51 51 TAGACAAACA CACACACACC AGATAATATA AACTTAATAA ATTATCTGTT
 101 101 AATGTAGATT TTATTTAAA AACTATATTG GAACATTGGT CTTCTTGGAA
 151 151 C

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TCU9/1(2)

5 1 ACATAACAGC TTTTATACAA TGATAAGGAC ATATCATTG TTTACAAAGA
 51 AAGTCTAAAA TTTCAAGAAC ATTCAAAGAG CTAACACAGT AAAGGTCACTG
 101 CAAGTTCTAG AATAGTGAAT CATGACAGAA CTCATTCTT TTATCCTTTA
 151 TCTCC

TCU9/2(2)

10 1 AAGTATGGGT AGCTAAATT GCATTAATT AAAAGTACAT ATAATGCAAC
 51 ACCACTCTAC ATCTGTATAC CTACGAATGT ATGTGTACTA CACACCCCTA
 101 AAATGTTTT CAAAGTCTTA ATATATTAGA ACATGTTTC ATTTTTCAT
 151 GGGATGTTAA TACTATTCTA TGATTAAGAA AATACTAG

TCU10(2)

20 1 AATACAGTTA TTCTAGCTTT TCATATTCAA TTTGAATGAT CAGAAAAGTA
 51 TATTAGTCAC ACAGAATTAA ATATTTAGA TAGTAAGAAT C

TCU14(1)

25 1 ATCCTTAGTA AGTGGATTTT GGGGAAAAAA GCACCTGGGC TTCTGGTTCT
 51 TTTTGATAAT ATATAAAATT ATTCAATTATG AGGTTGCAGT TGTTGCAAA

TCU14(2)

30 1 GAAGTGAAG TCAGCCCTTT AGCTATTATT TATTGTTTA TTAGAGCAGA
 51 GGGAAAGTGAC ACTCATTGCC TTCACAGAGC TCTGCAGAAA TATATGCACA
 101 GAGTGGTCAA TGCCAACATC TGAGTAAGTC TTCCAAA

TGO20(2)

35 1 CAGAACATTA GGATTTATTC CTTGATTAGT TCAAATGATT TCAACAGCTG
 51 AATTCCCTGA GATGTGTAAG GCAGGTTGGT CCTTTGGATG GACTGTAGAC
 101 TGAAACTTCC TATAACTGTA GTGATATGTA CACACCTACA TAGCAAAGTG
 151 CTTCATTATG AAAATGAAGA A

TGO20(1)

40 1 CAGTGTGAGA GTCTCATTTC TATGCACAGT GTTTCAGG AGGATGGAGC
 51 TAGTTAGCTG TCTGTTGTCT GTAGCCCAGC TTGATAATGG AACTATACAG
 101 CGAAGAGACA ATCTCTGGCA AGTTTTGTA GAA

TGU5(C)

45 1 TTAGAGTAAA ATTCCAAATA AATGCTTTGC TCCAAAATTA CACTAACCAAG
 51 GCTGGGTCTC TATCATACT CTTCAATACC CTCAAACCTA GATTGTAAAG
 101 TGAAAAAAAGT GATTAGCNNT TCCATTGTT CATTCTGTCA CTCACATTCT
 151 TAGGCATTTT AAGGATGAGC AACCTTTGTT TCAGAAAGGG TAAGTAATTAA
 201 GCCCCCTGGA GGTACATAG TTATAATTAA GTCTTCAGAA TCCGTTCGAA
 251 GGGNNNNNGTT ACTATTTTTA AGATAATTAG AACCCACCTT GTAGCAATAA
 301 AAGTTTCTT GTCTTTG

TGUS(2)

5 1 GCGAAAGACT AATCGAACCA TCTAGTAGCT GGTTCCCTCC GAAGTTCC
 51 TCAGGATA

TGU9/1(2)

10 1 TTAATGTTA AATACTACTT TTTTTCAAG CTTGCCCTAG ATACCAACTG
 51 TTTATCTAAC ACACAATTCC AGTGTGCCA AGCCTCATGC CAATTGAG
 101 GGAACAGCCA AAACTTATGC ATTCAATATAA AAAGAGTCTC TAGGCTCTTA
 151 TATCTACATT ATAATTTT

TGU9/2(2)

15 1 GGAATAACAT TTTTTATGA GGGAACCTT TAAAATGGAT GCACACAGTG
 51 GCATTTCTC CTAGGCTCAA AGCTGAGTAC ACTCCCGTAA TTTAATAAT
 101 ATTTTAGGCA AGTCCTATGA CAATTATACC AACAAAGTTTC TTCAACCCCA
 151 CCACCACCCC ACCATCTCTA TGC

TGU12(C)

25 1 GGAGGAAGCT TTATTTGGGA AGAGTGCAGGT TCNNTCGGCC CTGATCAGCT
 51 CTAGCCTGCC CACCCCATCT CAGCCAGGCG GCTTTACTTC TTCCCTGAGCT
 101 TCAGGTCTTT CTTCTCCTG ATTTCCCTGG CCAGCTCCCC AATCAATCTC
 151 CAGTACTCAT TGAACATTGAG CTCCGAGNCC TGATTACAT CCAAGCTCTT
 201 CATCTTCT

TGU13/1(C)

30 1 GGATGTGGTA GTTGATCTTT AATGCCATT CTAGGTCGGA AAAATCCATG
 51 ATCCTAACTT TTAAGAGAAG GTGGTAACT CTACTTAGGA CTTTTTTTG
 101 TAAGAGGAAT AATGTAGCCT CACCCCTTATC TTTCTGGAAA TGTTTAAACC
 151 ACTGAAATAT GGAGATCAAA TCCAGCTTAC ACACTGGTAA CTCAAATACT
 201 ATTTTTTTTT TAAACTATCT TTTCTAAACT AATCACCCCT CTTGTACATA
 251 GAACTTTCTA TCTCAGTGCC AATTCTTAGA CGTTGATGCA AACAGCTCTC
 301 CAGAGAGCCT GTGCTATTGT TC

TGU13/2(2)

40 1 GGGGTGTACA TTTTATTGGA AACCTTAAAT ACTGTCAGA AAGAATATAT
 51 CTTCAATCAA GGTCTGCCG AGCCTACACA GAAAAATGAA GCTTTTGGG
 101 TTAGGGCAA GGATATATAC AGTACAGAGG ACAAAAGA

TTO16/2(C)

50 1 ACATTCACTT AAGATGAAC TTCAGCATCT TCACCTGAG ATCCATCAGA
 51 TGATTCTGAG AGGCAGGTCT CCCCCAAAAA TCCACCGCAT GTATTCTTTC
 101 GTTTGAATC TGAAAGCCTC TTTCTTTCA GGCTTGATGA CTCTTCTAAG
 151 GTATTTGTTA TGCCCTCTT CTGGGTTTTT CGTTTGCCT TATCAAGTAG
 201 CTNAAATTCA AACACCAGGG CAANAGAAAC TGCTTCTAT

TTO20/1(C)

5 1 CCACCAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTCAC AGTGAGATAT
 51 TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT
 101 CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTTAACCTG
 151 GAGTTGATTA TACCATCACT GTGTATGCTG TCAC TGCCG TGGAGACAGC
 201 CCCGCAAGCA GCAAGCCAAT TTCCATTAAT TACCGAACAG AAATTGACAA
 251 ACCATCCCAG ATGCAAGTGA CCGATGTTCA AGACAACGT TTTAATAAAA
 301 GATTACATT CCAC

TTO20/2(2)

15 1 TTGGTACCAAC AGTCACAGAA CTGGGGGTCA TTTCTAGAT GAAACAAACG
 51 GAACAAGTTC TCTTCCAACA AAGAAATGTA CTGTAGAAAT TAATTTCCCTC
 101 CATGAATTTC ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA
 151 AG

TTU2/1(2)

20 1 CTAGAACCTTC CAAAGGCTGC TTGTATAGA AGCCATTGCA TCTATAAAGC
 51 AACGGCTCCT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAAGTC
 101 ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCAATGC TTCTCG

TTU 2/2(C)

30 1 AACAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAGAT
 51 TGTTTCTTA TCAGTAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG
 101 CATGTTTTC TTCATAGATA CCCGTTTGG GGTTTTGCG TCGGAAGATG
 151 AAGTGCAGTT TATAGTCCTC TCCACATTTA TCTG

TTU3(1)

35 1 GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATT CGG
 51 GAGGACCTAT TGGTGCAGGG GCTTGTATG ATTATGGCG TTGATTAGTA
 101 GTAGTTACTG GTGAACATT GTTTGTTGGT GTATATATTG TAATTGAGAT
 151 TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG
 201 AAG

TTU 5/1(2)

45 1 GACAAAAAAA AAAAAACAGG TTTTAAAGCT AGAAATGAAA AGCTACTTAA
 51 GTATCTTAA GGATAAGTTA CTTTATTATA CACTAGAAAC ATACACAATA
 101 GCTGAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG

TTU5/2(2)

50 1 GCATCCATTG TACATTGTTT GGTTTGAGGT TACCATGAGG CCTGTAATA
 51 CTATCTTATA ATTATTATT TCAACCTGAT AAAACTTAAC ACTATTTGCA
 101 TAAACAAACA AACGAAAA

TTU9/1(1)

5 1 TAAAAACTG GTTCTTTAT TCTGCAATAT TTTAAAAAT CACATTTCA
 51 GCCAGGCGCA GTTTCCACA CCTGTAATCC GGCACTTGG GAGGCTGAGA
 10 101 TGGGTGGATC ACAAGGTAGG AGATCAAACA TCCTGCCAA CATGGTGAAC
 151 CTGTTTACT

10

TTU9/2(2)

15 1 CAAGTATGGG TAGCTAAATT TGCATTAAT TAAAGTACA TATAATGCAA
 51 CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCCT
 101 AAATGTTCA AAGCTTAATA TATTAGAACCA TGTTTCATT TTCAGGGAG

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TTU13(2)

20 1 GGAAATACAC TAGCATGTGA GCACTGTATA TAAAGCTTGA GGTTAGGAGG
 51 TAAAATGAAA GAAATCATT TAAACTCCTA AGATGT

20

TTU13(1)

25 1 TGAATTAAAT GGACTCGTTG AAAGGACAAG GAGATCGGTA ATATCTCT
 51 AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT
 101 CTTCTTTT

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or an analog thereof.

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11. Vector containing a DNA according to claim 10.
12. Host cell containing a vector according to claim 11.
- 35 13. Method for isolating a gene inducible by treating chondrocytes with IL-1 β containing the steps:
 - (a) hybridizing a DNA according to claim 10 under stringent conditions against DNA or RNA containing said gene; and
 - (b) isolating said gene.

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14. A method according to claim 13 wherein said DNA or RNA has been isolated from chondrocytes, particularly human chondrocytes, that were treated with IL-1 β .
- 45 15. Process for expressing a gene isolated according to claims 13 or 14 containing the steps:
 - (a) cloning said gene into a suitable expression vector; and
 - (b) expressing said gene in a suitable host cell.

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16. Method for producing a protein containing the steps:
 - (a) culturing a suitable host cell containing a vector which contains a DNA according to claim 10 or a gene produced by a method according to claim 13 or 14; and
 - (b) isolating the expressed protein.
- 55 17. Diagnostic aid containing a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof.

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18. Use of a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof for the diagnosis, prophylaxis or therapy of IL-1 β mediated diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

5 19. Use of a gene isolated according to claim 13 to 14 for the production of a pharmaceutical.

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